

Selected Physiological Responses of *Salvinia minima* to Various Temperatures and Light Intensities

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ABSTRACT.—Two separate experiments were conducted to determine the influence of temperature (15, 23 and 35°C) and various light intensities, ranging from 80 to near 700 $\mu\text{mol}/\text{m}^2/\text{s}$ on selected physiological responses of salvinia (*Salvinia minima*). This was an attempt to determine the distribution range of this plant as influenced by these selected environmental factors. The first experiment was carried out for 14 days under controlled environments, with a light intensity of 120 $\mu\text{mol}/\text{m}^2/\text{s}$ and 14 h photoperiod. Plant growth was the highest at 23 and 35°C, in comparison to those grown at 15°C. The chlorophyll concentration was less influenced by the temperature than by the growth; however, carotenoid concentration at 35°C was significantly higher than those obtained from the plant grown at 15°C. Salvinia acclimation to cold temperature possibly included an increase in anthocyanin and soluble sugar concentrations. The second experiment was carried out under greenhouse conditions, 25–27°C and various light intensities ranging between 80 to near 700 $\mu\text{mol}/\text{m}^2/\text{s}$ in order to determine the light saturation curve. Salvinia was shown to have a wide range of acclimation ability to various light intensities ranging from 80 to near 700 $\mu\text{mol}/\text{m}^2/\text{s}$. This study should be helpful for determining the ecological distribution of salvinia.

KEY WORDS.—*Salvinia minima*, CO_2 assimilation, photosynthetic pigments, light intensities

The genus *Salvinia*, from the family Salviniaceae, is comprised of 12 known species (Nauman, 1993). *Salvinia minima* Baker is a small, free-floating freshwater fern found in tropical and temperate regions (DeBusk and Reddy, 1987) and in areas such as North, South, and Central America, the West Indies, and Africa (Nauman, 1993). This species is commonly referred to as water fern and South American pond fern (Nauman, 1993). In the United States, salvinia was first discovered in the St. John's River, Florida in 1928 (Long and Lakely, 1976) and was speculated to have been introduced by accident through its discharge from contaminated boat ballasts from South American ships or from accidental release from aquarium sources (Schmitz *et al.*, 1991). This plant can be found floating near the edges of slow moving streams and in nutrient enriched ponds. Salvinia forms a "mat" that covers very large portions of the body of water where it grows. Salvinia reproduces exponentially by vegetative fragments, with a leaf doubling time of 3.5 days (Nichols *et al.*, 2000). The rapid growth of salvinia has been recognized as an ecological problem in many southern coastal regions of the United States. This is due to the impact on suppressing the growth of native vegetation and the degradation of water quality; this includes reducing oxygen concentration, reduces the light

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penetration, and other ecological impacts (McFarland *et al.*, 2004). However, The unique characteristics of salvinia, which include rapid growth, the high acclimation capacity of the plant to wide range of temperatures, and relative tolerance to a wide range of contaminants make it a prime candidate for phytoremediation (Olguin *et al.*, 2003; Olguin *et al.*, 2007). For instance, *Salvinia minima* demonstrated the ability to withstand aluminum (Al) concentrations of 20 mg/l through the manipulation of the media pH from 3.9 to near 7 within 24 hours (Gardner and Al-Hamdani, 1997). In addition, salvinia has the ability to survive and grow under highly eutrophic environments unsuitable for other species found in similar environments such as azolla (*Azolla caroliniana* Willd.) and duckweed (*Lemna minor* L.) (Reddy and DeBusk, 1985).

Whether salvinia is perceived as either a noxious or beneficial weed, it is essential to determine the environmental requirements for its growth. Temperature and light intensity are among major environmental factors determining the ecological distribution of any plant (Larcher, 2003). There is a lack of general information regarding these environmental requirements for salvinia growth. Therefore, in this study, the influence of selected temperatures (15, 25, 35°C) on salvinia growth was determined. To examine the ability of salvinia to combat the impact of low temperature, the accumulation of soluble sugar was examined. The accumulation of soluble sugar is one of the major defense mechanisms of plants to cold temperatures (Larcher, 2003). In addition, the photosynthetic light response curve was determined to evaluate the photosynthetic response of salvinia to different light intensities. The photosynthetic pigment concentrations at different temperatures were evaluated and anthocyanin concentration was also determined. Anthocyanin is a major flavonoid pigment which is considered a free radical scavenger reducing the damages resulting from oxidative stress such as photooxidation (de Pascual-Teresa and Sanchez-Ballesta, 2007).

MATERIALS AND METHODS

To accomplish the objective of this study, several separate experiments were carried out under controlled environments of temperature and light intensity. To determine the effect of temperature (15, 23, 35°C) on salvinia growth, a total of 20 fronds were placed into 250 ml Erlenmeyer flasks containing 125 ml of 10% Hoagland's solution (Hoagland and Arnon, 1938). The plants utilized in this study were taken from stock material growing under greenhouse conditions. The plants of all treatments were grown for fourteen days in a growth chamber at 23°C, 120 $\mu\text{mol}/\text{m}^2/\text{s}$ photon flux density and 14 h photoperiod. Twelve flasks (samples) per treatment were used. The samples of each treatment were placed in separate containers. Water was added to each container, and the flasks were partially submerged in a water bath. The individual temperatures were controlled by passing water with the desirable temperature from a circular water bath unit through cooper coils located inside the individual water containers.

The plants of six samples (flasks) from each treatment were used for growth determination. The remaining six samples of each treatment were used to determine the soluble sugar content. *Salvinia* growth was determined using fresh weight and frond number doubling time. Frond number of each sample was counted at the initial day of the experiment (day1) and on day 7 and 14. The frond numbers were used to evaluate the growth rate using the following doubling time formula: $DT = t \log 2 [\log (w_t w_o^{-1})]^{-1}$ (Moretti and Gigliano, 1988) where DT is the doubling time (days), t is the experiment duration (days), w_t is the final number of frond, and w_o is the initial frond number. Approximately 0.1 g fresh weight of each sample was used for measuring chlorophyll a and b , and carotenoid concentration. The plants were placed into 5 ml of N,N-Dimethylformamide (DMF) solution. The samples were incubated in the dark for 36 hrs at 4°C. Chlorophyll a and b was determined spectrophotometrically at wavelengths of 647 and 664.5 nm (Inskeep and Bloom, 1985). The anthocyanin was determined by homogenizing 0.1 g fresh tissues in 5ml methanol containing 1% HCl (v/v) for 2 min on ice. The homogenate was filtered and absorbance of the extract was determined spectrophotometrically by the method of Mancinelli (1990).

Soluble sugar analysis was conducted following a procedure slightly modified from Chatterton *et al.* (1987). Six randomly selected samples of each treatment were oven dried at 65°C for 48 h. The dry samples were ground into a fine powder, and a 100–500 mg portion was placed in a sealed vial and used to measure soluble sugars as reported in detail by Wilson and Al-Hamdani (1997).

This experiment was repeated twice each and statistically analyzed as a randomized complete block design (Steel and Torrie, 1980). This design ensured that observed differences in plant performances were largely due to treatments rather than variation among the four blocks (experiments conducted at different times). Mean separations for the treatments with significant F values ($P = 0.05$) of ANOVA analysis were based on the least significant difference (LSD) test (Steel and Torrie, 1980).

The second experiment was carried out to determine the photosynthetic light response curve. The *salvinia* was grown in Hoagland's solution, as in the first experiment. Six flasks containing 20 fronds of *salvinia* were grown for 7 days under greenhouse conditions of 25–27°C and various light intensities, depending on the time of day, ranging from 80 to near 700 $\mu\text{mol}/\text{m}^2/\text{s}$.

Carbon dioxide assimilation of the six samples was measured starting four hours after the onset of the light period at day seven. The selected plants of each sample were placed on wet filter paper and enclosed in a flow-through plexiglass assimilation chamber (4.5 by 11.8 by 7.3 cm) of a Li-Cor 6200 photosynthesis system (Lincoln, NE, USA) as described by McDermitt *et al.* (1989). Standard measurement conditions were 27°C, various light intensity with ranges from 80 to near 700 $\mu\text{mol}/\text{m}^2/\text{s}$ photon flux density and 75% relative humidity. To establish a light saturation curve, photosynthesis measurements were taken at various times of the day to obtain the desired range of light intensities. This experiment was repeated three times.

TABLE 1. The effect of different temperatures on salvinia growth and soluble sugar accumulation.

Temperature (°C)	Doubling time (days)		Soluble sugar* mg/g dry weight
	Length of exposure (days)		
	7	14	
15	−33.559a	53.364a	78.130a
23	3.865b	4.668bb	33.830b
35	3.468b	7.741b	14.953b

Mean within the column followed by the same lower case letter are not significant based on LSD ($P = 0.05$).

* The soluble sugar was determined after fourteen days of exposure at the selected temperatures.

RESULTS AND DISCUSSION

Assessment of salvinia growth at the three selected temperatures clearly showed that exposure to colder temperatures (15°C) resulted in significant growth decline in contrast to samples exposed to higher temperatures (Table 1). After seven days of growth at 15°C, salvinia doubling time showed a negative value, indicating no determinable change in the growth status during that period. This result was expected since salvinia is a tropical plant and is susceptible to cold temperatures (Gaudet, 1973). Debusk and Reddy, 1987 reported that the growth rate of *Salvinia rotundifolia* was significantly lower at 10°C, compared to samples grown at 25°C. The optimum growth temperature for most tropical plant species was reported between 23 to 32°C. (Lee *et al.*, 2007). Salvinia grown at 23 and 35°C showed similar rapid growth, as indicated by the doubling time values, which was less than four days (Table 1). The growth results after 14 days were comparable to those at seven days. The exception were the plants grown at 15°C, which demonstrated very slow growth rate, with a positive value for the doubling time (53.36 days). This result indicates that salvinia can survive at low temperature of 15°C.

Chlorophyll *a* and *b* concentrations were similarly influenced by the three temperature treatments (Table 2). The exception was plants grown at 35°C which showed significantly higher chlorophyll *a* concentration than the other temperatures. Similar findings were reported by McWilliam and Naylor (1967). They reported a reduction in chlorophyll content in corn (*Zea mays* L.) associated with lowering the growth temperature from 28 to 16°C. The commonly observed reduction in chlorophyll concentration in tropical plants at low temperature was attributed in part to an aberrant development of the thylakoid membranes (Hodgins and van Huystee, 1986). In addition, low temperature was found to induce a reduction in several enzymes associated with chlorophyll synthesis in the plant (Tewari and Tripathy, 1998). In this study, the temperature treatments of 35°C induced significant increases in carotenoid concentration in comparison to those grown at 15°C (Table 2). However, temperature effect of 23°C on carotenoid concentration was not significantly different to those grown at 15 and 35°C. Lefsrud and Kopsell (2005) reported an increase in β -Carotene concentration in kale (*Brassica*

TABLE 2. The influence of selected temperatures on chlorophyll a (chl a), chlorophyll b (chl b), carotenoid and anthocyanin concentrations in salvinia after fourteen days of growth.

Temp. (°C)	Chl a (mgg ⁻¹ fr.wgt.)	Chl b (mgg ⁻¹ fr.wgt.)	Carotenoid (μgg ⁻¹ fr.wgt)	Anthocyanin (μgg ⁻¹ fr.wgt)
15	6.163a	3.747a	515.075a	31.480a
23	6.762a	4.235a	686.625ab	10.478b
35	8.903b	4.963a	1252.452b	21.698c

Mean within the column followed by the same lower case letter are not significant based on LSD (P = 0.05).

oleracea L.) in response to gradual temperature increases from 15, 20, 25 to 30°C. Similarly, Leipner *et al.* (1997) found a decline in chlorophyll *a* and chlorophyll *b* and carotenoid concentrations in corn associated with lowering the temperature from 25 to 15°C.

Anthocyanin concentration was significantly different among the plants grown at different temperatures (Table 2). The highest anthocyanin concentration was obtained from the plant grown at 15°C, followed in decreasing order by those grown at 35 and 23°C. The increase in anthocyanin concentration in response to the relatively low (15°C) and high (35°C) temperatures might be considered an acclimation response to stress. This conclusion was also supported by Doong *et al.* (1993), who reported that anthocyanins are produced by most aquatic plants in response to stress factors such as high light intensity, high temperature, or nutritional limitations, and can be used as a stress indicator. Increased anthocyanin concentrations were also found to be induced in azolla by Al stress (Ayala-Silva and Al-Hamdani, 1997) and by Cr (VI) (Wilson and Al-Hamdani, 1997).

Salvinia accumulation of soluble sugar was significantly increased with each decrease in temperature from 35 to 23 and 15°C (Table 1). This increase in soluble sugar might represent an acclimation response to low temperature. Sugar accumulation could play an important role in combating the influence of low temperature (Levitt, 1980; Hurry *et al.*, 1995). Similar findings were reported by Al-Hamdani and Thomas (2000). Strand *et al.* (1997) suggested that an increase in soluble sugar accumulation is an essential response to combat the cold temperature stresses. The advantage of increasing soluble sugar is associated with the reduction in freezing point and increase plant tolerance to cold temperature (Nilsen and Orcutt, 1996).

The connection between light intensity and photosynthesis showed that the light-limiting portion of the light saturation curve extended to near 300 μmol/m²/s (Fig. 1). This can be used as an indicator that photosynthesis was operating linearly with the light intensity during this portion of the curve. The CO₂ limiting portion of the curve extended from 350 to near 700 μmol/m²/s. Similar findings were reported for Floating Pennywort (*Hydrocotyle ranunculoides* L.f.) with a light saturation of CO₂ gas exchange between near 350 to near 800 μmol/m²/s (Hussner and Losch, 2007).

In conclusion, salvinia growth was the highest at 23 and 35°C and lowest at 15°C. However, the reduction in plant growth was not severe enough to totally

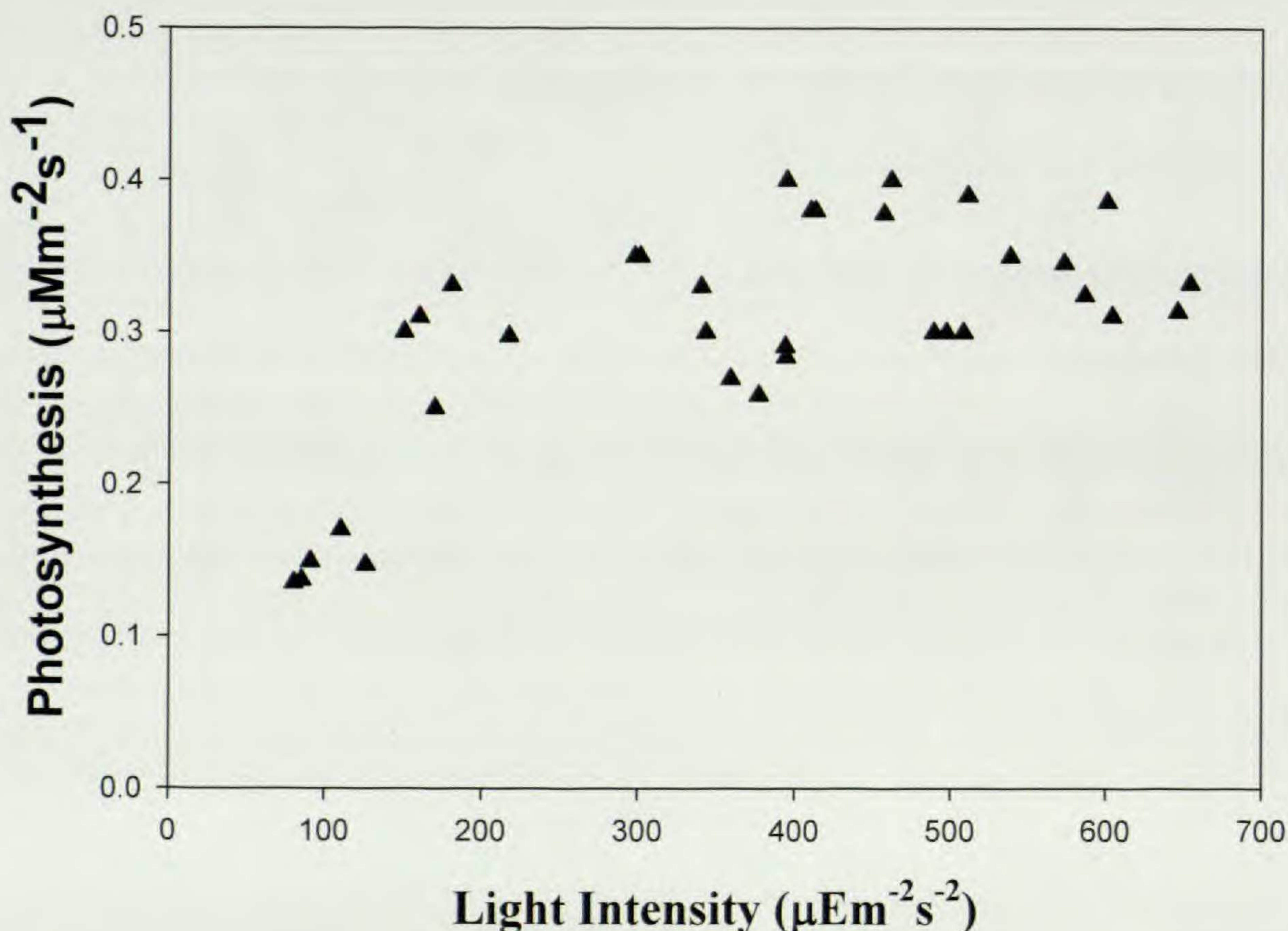


FIG. 1. The relationship between photosynthesis and light intensity for *Salvinia minima*.

inhibit plant reproduction. *Salvinia* acclimation to cold temperature possibly included the increase in anthocyanin and soluble sugar concentrations. The light saturation curve indicated that *salvinia* has a wide range of acclimation to various light intensities ranging from 80 to near 700 $\mu\text{mol}/\text{m}^2/\text{s}$ and should be helpful for determining the ecological distribution of *salvinia*. Since one of the major criteria in selecting plants for ecological restoration is the acclimation capacity to the range of temperature and light intensity (Salt *et al.*, 1998), *salvinia*'s capability to withstand a diverse range of environmental variables make it a prime candidate for phytoremediation.

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